Detection of Pepsinogen II in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
DAB Chromagen
Hematoxylin

Primary Antibody: Sheep Polyclonal to Pepsinogen II
Abcam, Inc
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab9013

Negative Control Serum: Normal Sheep Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 013-000-001

Secondary Antibody: Rabbit Anti-Sheep IgG (H+L) Biotin-SP-Conjugated
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 313-065-003

Label Complex: R.T.U. Vectastain Elite ABC Reagent
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Gastrointestinal tract - stomach
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Block with 10% bovine serum albumin for 20 minutes at room temperature.
   Lot #_____ Exp. Date_____

   DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

5. Avidin / Biotin Blocking Kit
   Lot #_____ Exp Date_____ New Kit: yes / no
   Apply avidin block for 15 minutes at room temperature.
   Quick rinse in 1X Wash Buffer.
   Apply biotin block for 15 minutes at room temperature.

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BUFFER.

6. Apply the primary antibody at a 1:25,000 dilutions and incubate for 30 minutes at room temperature.
   Lot #_____Exp. Date_____

   For negative control slides, dilute the protein concentration of the normal sheep serum to match that of the primary antibody. Make a 1:25,000 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.
   Lot #_____ Date Reconstituted_____

7. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

8. Apply the rabbit anti-sheep secondary antibody at a 1:1000 dilution. Incubate for 30 minutes at room temperature.
   Lot #_____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

10. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
    Exp. Date_____ New Kit: yes / no

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

12. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.
    (Add 1 drop of DAB per ml of substrate)
    Lot #_____ Exp Date_____ New Kit: yes / no

13. Rinse the slides in tap water 3 minutes.

14. Counterstain with Harris Hematoxylin for 20 seconds.

15. Rinse the slides in tap water until water is clear.

16. Gently agitate slides in 1X Wash Buffer until the tissues turn blue.

17. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

18. Coverslip

Updated 12/28/11